Pulmonary intravascular macrophages and endotoxin-induced pulmonary pathophysiology in horses

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Abstract

Endotoxemia causes significant mortality and morbidity in horses. The mechanisms underlying this complex pathophysiology remain unclear. Therefore, effective tools to treat endotoxemia in horses are lacking. Furthermore, the multifactorial and multiorgan pathophysiology of equine endotoxemia has not been fully addressed, especially the lung injury associated with endotoxemia. Within the context of the broader picture of endotoxemia and lung injury, we offer a perspective on the roles of pulmonary intravascular macrophages in endotoxin-induced lung inflammation in horses.

Résumé

L'endotoxémie est une cause de mortalité et de morbidité important chez les chevaux. Le mécanisme qui sous-tend cette pathophysiologie complexe demeure nébuleux. Ainsi, des outils efficaces pour traiter une endotoxémie chez des chevaux sont manquants. De plus, la pathophysiologie multifactorielle et multi-organes de l'endotoxémie équine n'a pas été complètement étudiée, plus spécifiquement les lésions pulmonaires associées avec l'endotoxémie. Dans le contexte plus global de l'endotoxémie et des lésions pulmonaires, nous offrons une perspective sur les rôles des macrophages pulmonaires intra-vasculaires lors d'inflammation pulmonaire chez les chevaux.

(Traduit par Docteur Serge Messier)

Bacteria and their products, such as endotoxins, cause many inflammatory diseases, such as endotoxemia. Endotoxemia is one of the leading causes of mortality and morbidity in adult horses and foals (1). Endotoxemia is referred to as "inflammation gone awry" (2). A major factor contributing to the inappropriate inflammatory response is the shedding of lipopolysaccharides (LPS) from gram-negative bacteria into the circulation. This pro-inflammatory molecule activates macrophages, neutrophils, and the endothelium of various vascular beds (3). Endotoxins have a critical role in equine diseases, such as acute abdominal disease, adynamic post-operative ileus, laminitis, and neonatal septicemia (4). Earlier data showed that approximately 80% of horses experience colic during their lifetime and a mortality rate of 40% was reported among 2500 horses that had colic and were referred to 12 university hospitals in the United States from 1981 to 1984. Furthermore, the severity of the intestinal lesion as well as the prognosis is directly correlated with the degree of endotoxemia (2). Recently, more data showed that the incidence of colic in different horse populations varied from 3.5 to 10.6 episodes of colic per 100 horses per year and the case fatality rates as a result of colic vary from 6.7% to 15.6%, depending on the population studied and the type of lesion; in some equine populations this is the single most common cause of death (5). These data support the notion that endotoxemia is a significant cause of economic damage to the equine industry.

Horses are unique in their extreme sensitivity to endotoxin-induced cardio-pulmonary shock and mortality. The intraperitoneal lethal dose of LPS for ponies is 200 to 400 μg/kg of bodyweight (BW), whereas the lethal dose for rabbits and guinea pigs is 3 and 10 mg/kg BW, respectively (6,7). The mechanism of these phenomena remains largely unknown (8). Thus, we describe how the pulmonary intravascular macrophages (PIMs) contribute to the endotoxin-induced lung pathophysiology in horses.

Recent data on the biology of Toll-like receptors (TLR) have enhanced our understanding of innate host responses to pathogens. It is now known that in addition to CD14, TLR4 is essential to ligate and phagocytose LPS (9). The ligation, phagocytosis, or both of LPS leads to activation of cell signalling, resulting in NF-κB activation and elaboration of proinflammatory cytokines (10). Because TLR4 is expressed on macrophages, monocytes, endothelial cells, and neutrophils (3), the consequences of endotoxemia are systemic vascular inflammation, as well as disseminated intravascular coagulation (11). Thus, the cascade ensued may lead to a dysregulated inflammatory response, such as that seen in endotoxemia or sepsis, leading to multiple organ failure, including cardiopulmonary shock.

The progression of endotoxemia to lung injury and respiratory distress is complex and follows multiple interrelated pathways. One proposed pathway is the uptake of endotoxin by the endothelial cells through molecules such as TLR and CD14 in the pulmonary vasculature, which causes direct toxicity to the cells (12). The other proposed pathway is the activation of the complement cascade by endotoxins, particularly C5a and C3a, which are anaphylatoxins and cause an increase in vascular permeability via mast cell degranulation. The endotoxin C5a further activates the lipoxygenase pathway in neutrophils and monocytes, acts as a chemotaxin for leukocytes,

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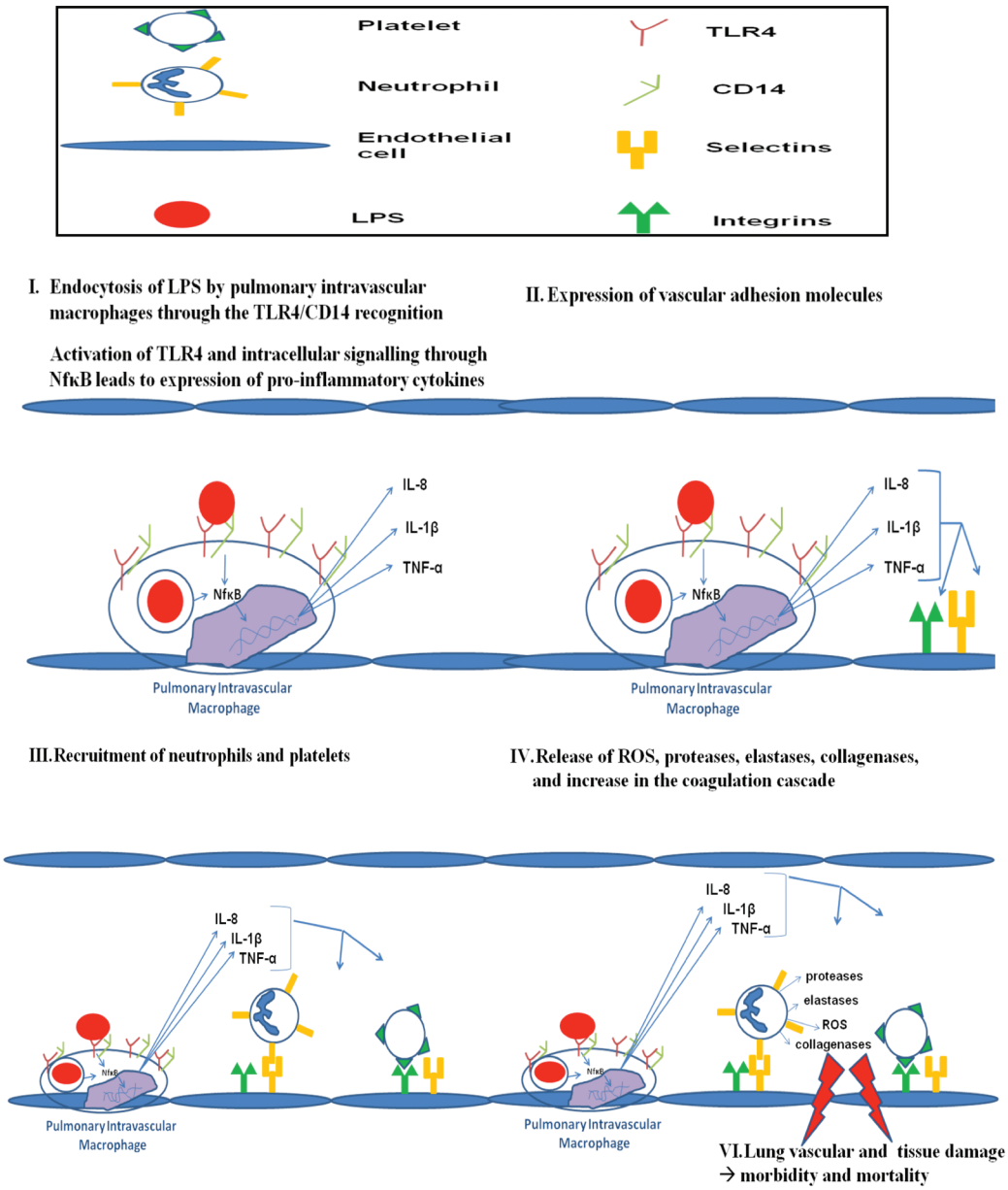

Figure 1. The role of pulmonary intravascular macrophages (PIMs) in the development of lung injury following endotoxemia. Stages I to IV summarize the known data such as the expression of Toll-like receptor-4 (TLR4), the uptake of lipopolysachharides (LPS), and localization of IL-1β and TNFα in PIMs of LPS-treated horses. The pathways still to be determined include upregulation of vascular adhesion molecules and migration of neutrophils in the lungs of LPS-treated horses.

and promotes neutrophil adhesion to endothelial cells, which subsequently causes activation of neutrophils and their accumulation in the pulmonary microcirculation (12–14). Activated neutrophils live longer and are believed to cause damage to vascular endothelium. The damaged endothelium in pulmonary microcirculation leads to increased permeability and development of lung edema.

Macrophages develop from monocytes and reside in various anatomical compartments in the lung. For example, in addition to well known and characterized alveolar macrophages, lungs also contain interstitial, airway, and intravascular macrophages. One of the earliest reports showing presence of PIMs in calf lungs was published in 1974 (15) followed by the evidence of erythrophagocytosis by PIMs in goats (16) and other species (15–22). The PIMs in the equine lungs were first described by Atwal et al (17) and subsequently Longworth et al (18). There are reports of smaller populations of PIMs occurring in rabbit and cat lungs as well (19). In postnatal pigs and sheep, the PIMs are sequestered within 4 wk of birth (20,21). However, there are no data on the ontogeny of PIMs in horses. Currently, there are no molecular or evolutionary explanations for the intriguing recruitment of PIMs in the host animal species and their absence in humans and rats.

It has been known for many years that PIM-containing animal species are more prone to lung inflammation (22). For example, an intravenous injection of 0.003 to 1.3 μg of *Escherichia coli* LPS/kg BW induces pulmonary hypertension and cardio-pulmonary shock in horses (22). A conclusive link was established between PIMs

and pulmonary responses in sheep. Neonatal lambs have very few PIMs and show no hemodynamic response to Monastral blue or liposomes (23). However, PIM sequestration in lambs by 2 wk of age is accompanied by enhanced vascular response to the same treatment; this hypertensive response is eliminated by removing the PIMs. Monastral blue is a copper dye that has been used as a phagocytic tracer to study PIMs in cattle, sheep, and horses (17). It is suspected that intravenous infusion of Monastral blue may activate PIMs to cause transient vascular response. Horses show transient pulmonary response following Monastral blue injection and horse PIMs are highly phagocytic for Monastral blue and develop large phago-lysosomes containing the dye following single or multiple intravenous injections (17,24). Multiple injections of Monastral blue results in larger phago-lysosomes compared to the single injection, which indicates that phagocytic capacity of PIMs is not diminished following initial round of phagocytosis of the dye (24). Pulmonary arterial hypertension observed in horses with intravenous injection of *E. coli* is blocked in animals in which PIMs have been depleted or inactivated (8,25). Endotoxin-induced pulmonary vascular responses are generally attributed to production of vasoactive substances, such as thromboxanes by the PIMs. This contention is supported by in vitro data which showed that porcine PIMs produce more arachidonic acid metabolites compared to the alveolar macrophages (26,27). Taken together, the evidence shows a causal relationship between PIMs and endotoxin-induced pulmonary vascular responses.

Macrophages and other cells engage endotoxins and other bacterial products through molecules such as TLR and CD14 (28). Recently, the first data on the expression of TLR4 in PIMs in horses (29), cattle and pigs was reported (29,30). Toll-like receptor-9, which engages bacterial DNA, has been observed in equine PIMs (31). Toll-like receptor-4, expressed in PIMs, is a major component of total TLR4 expression in horse lungs because depletion of PIMs significantly reduced lung expression of TLR4. It is interesting to note that total lung expression of TLR4 protein and mRNA was significantly reduced following depletion of PIMs. These data show that, although lung epithelial and endothelial cells express TLR4 and TLR2, PIMs are the major source of these innate immune receptors. The role of TLR4 is to engage and endocytose endotoxins and initiate cell signalling via NF-κB pathway, which results in cytokine expression. Previous data showed an association between TLR4 and *E. coli* LPS, as both molecules were co-localized in PIMs of horses treated with the LPS (29). Therefore, a decline in TLR4 in lungs of PIM-depleted horses may result in reduced sensitivity to bacterial products. The strategic vascular location of PIMs, their significant phagocytic ability, and the TLR4-LPS colocalization uniquely enables these cells to sense and respond to circulating endotoxins in conditions such as endotoxemia and subsequently to initiate a pulmonary response (Figure 1).

It is known that the interaction of an endotoxin with a TLR4 or the uptake of LPS by macrophages activates macrophages (32). Once activated, macrophages, including TLR4-expressing PIMs, secrete mediators, such as IL-1β and TNF-α, to induce expression of adhesion molecules on lung microvascular endothelium (29,33,34). Furthermore, secretion of vasoactive substances, such as thomboxanes and inflammatory cytokines (IL-1β, TNF-α) by the PIMs into the circulation will affect lung vascular endothelium to initiate lung inflammation, as reported in horses and other species (35). Horses treated with *E. coli* LPS showed increased expression of TLR2 mRNA (29) and TLR9 (31) in the lungs, which will potentially alter PIM responses to other bacteiral ligands, such as lipotechoic acid and CpG containing motifs. Although it is well recognized that the lung is affected in sepsis (36), lung inflammation is not considered to be a major complicating factor in equine endotoxemia. If this contention is indeed true, it still is possible that cytokines secreted by the activated PIMs into the circulation may affect other organs. Endotoxemia is an important risk factor for development of acute laminitis in horses (37). Local digital cytokine gene expression and infiltration of the laminar tissue with leukocytes was suggested to be one of the causes of laminitis (38). A remote production of cytokines and vasoactive substances by the PIMs and their secretion into the circulation during endotoxemia would possibly reach the hoof and achieve similar effects.

The involvement of PIMs in equine pulmonary pathophysiology is not limited to endotoxemia. It has been suggested that the changes in pulmonary microvasculature and the subsequent edema seen after infection with African Horse Sickness virus is partially due to the activation of PIMs and the subsequent release of chemical inflammatory mediators (39). Furthermore, exposure to inhaled halothane was shown to induce translocation of the PIMs surface coat into the vacuolar system of the PIMs, followed by enrichment of acid phosphatase in the Golgi apparatus, and a development of extensive lamellipodial extensions. These extensions were suggested to enable the PIMs to interact with platelets within the pulmonary capillaries, forming thrombotic aggregates of platelets (40).

Currently, we know the structure of equine PIMs, their phagocytic abilities and some aspects of their inflammatory phenotype. The data from other species suggest that PIMs depletion may have beneficial effects for the host. However, similar detailed information is still not available on the equine PIMs. Based on the totality of information on the biology of PIMs, we believe that PIMs play a significant role in vascular inflammation in the horse (Figure 1) and merit further investigations in complex conditions such as endotoxemia.

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